DESIGN AND FORMULATION ASPECTS OF MONOCLONAL ANTIBODIES FOR SARS-COV-2 TREATMENT

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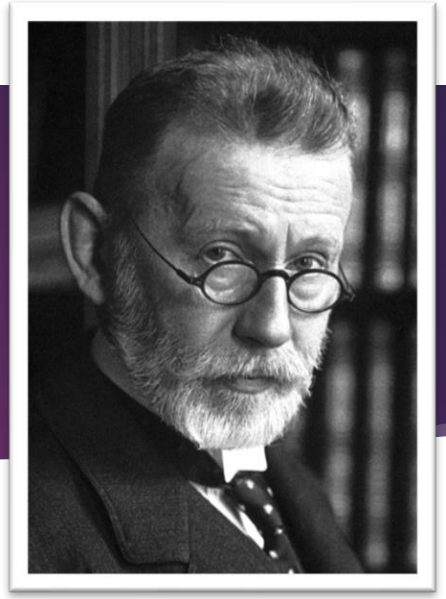
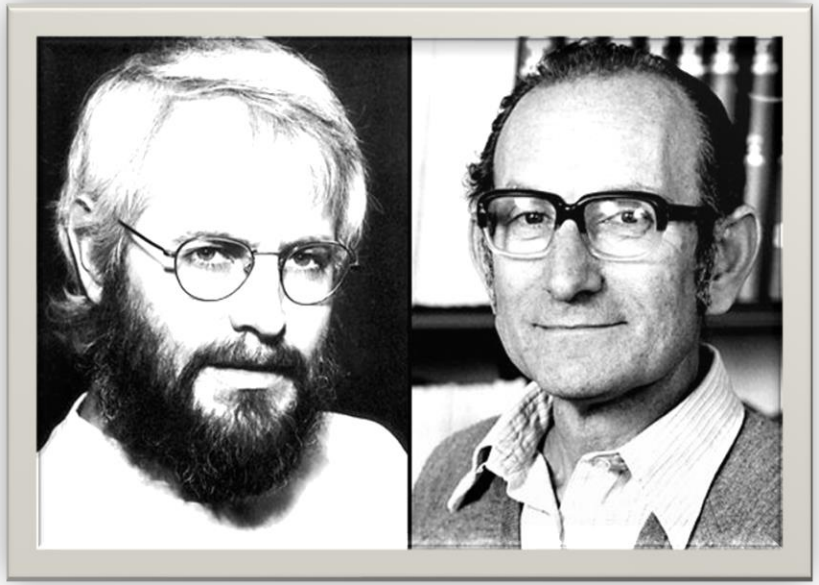
Montenegrin International Medical Summit, Podgorica, 14-17.10.2021

Paul Erlich
1854 - 1915
Nobel Prize for Physiology or Medicine in 1908



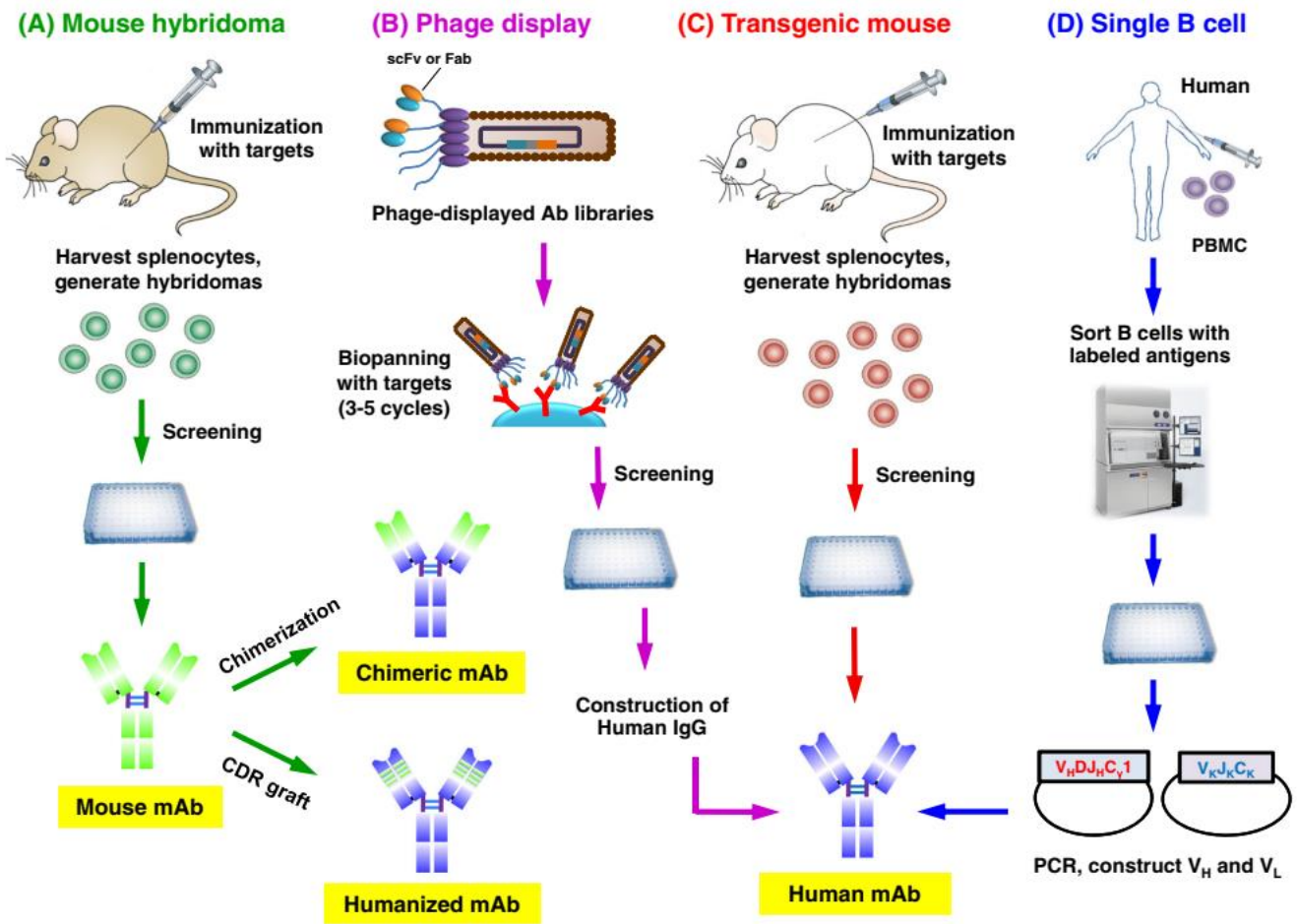
- Side chain theory of immunity
- Antibodies - magic bullets 1899/1906



Georges J.F Köhler and Cèsar Milstein
Nobel Prize for Physiology or Medicine in 1984

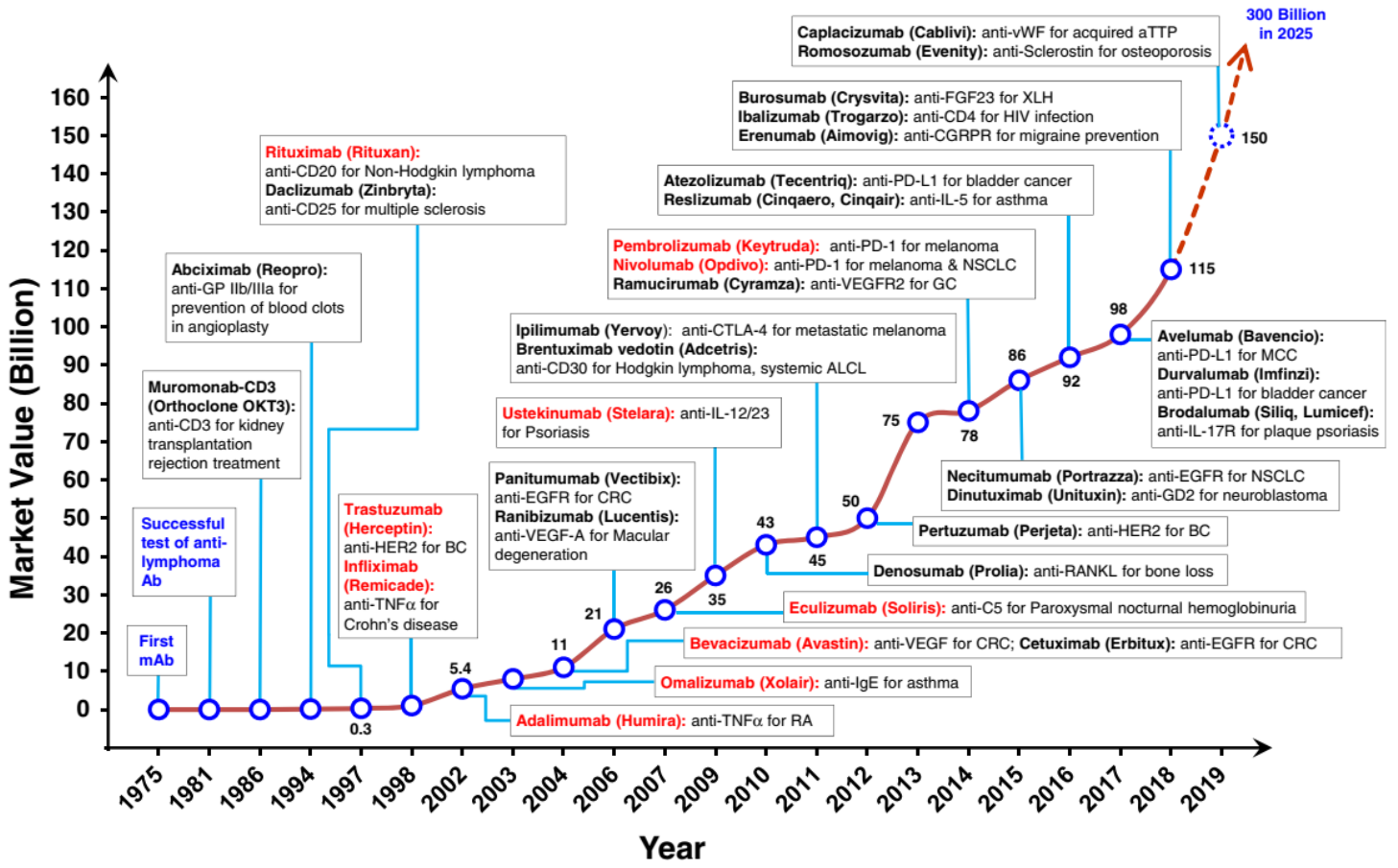
- 1975 introduced hybridoma technology
- the first monoclonal to be approved (OKT-3®, muromonab-CD3) was approved in 1986 for a single treatment regimen to protect from life-threatening tissue rejection following kidney transplant

Design of monoclonal antibodies (mAbs)



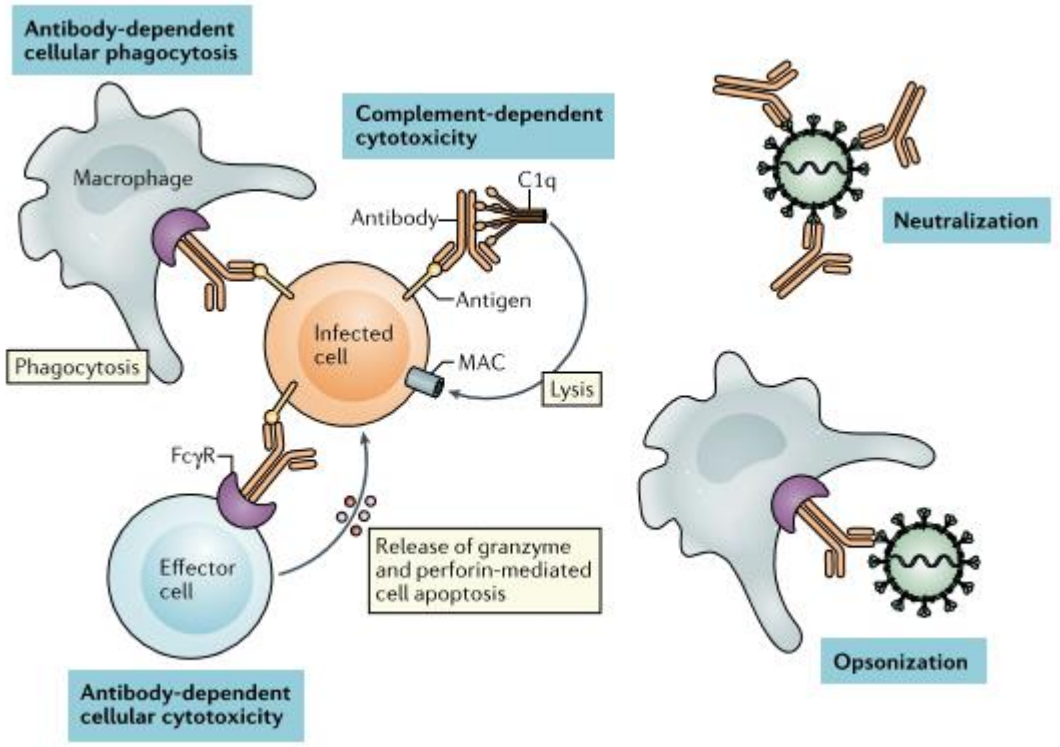
J Biomed Sci **27**, 1 (2020)

mAbs industry is constantly growing



J Biomed Sci **27**, 1 (2020)

mAbs mechanism of action



Directly interfere with (viral) pathogenesis

- binding of a neutralizing antibody to the virion can prevent target cell binding and/or fusion
- antibody binding opsonizes the virions or infected cells for phagocytic uptake
- mAbs can facilitate target cell death via
- complement fixation and membrane attack complex (MAC) activation
- antibody-dependent cytotoxicity

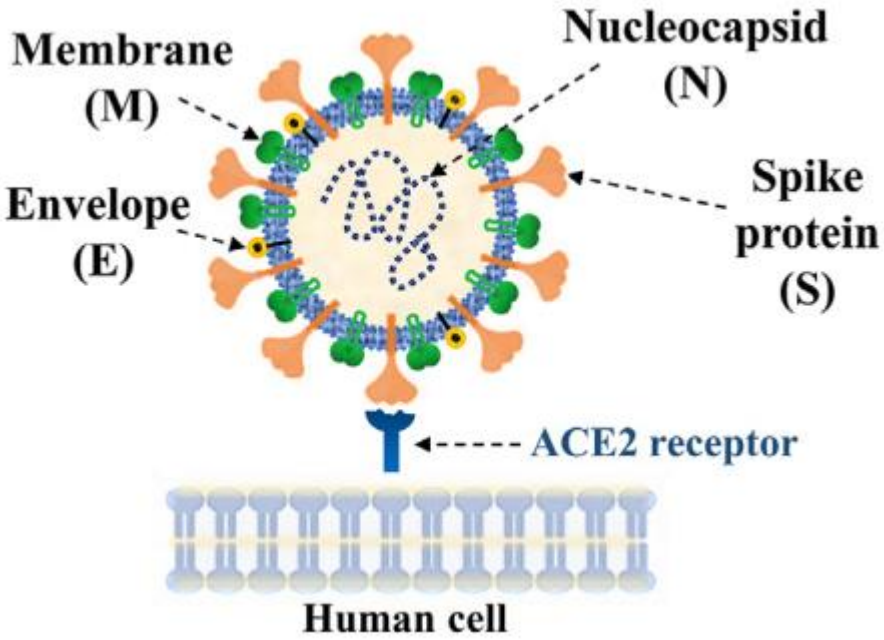
resulting in apoptosis or necrosis of the infected cell

Nat Rev Immunol **21,** 382–393 (2021)

Antigenic target for SARS-CoV-2 virus

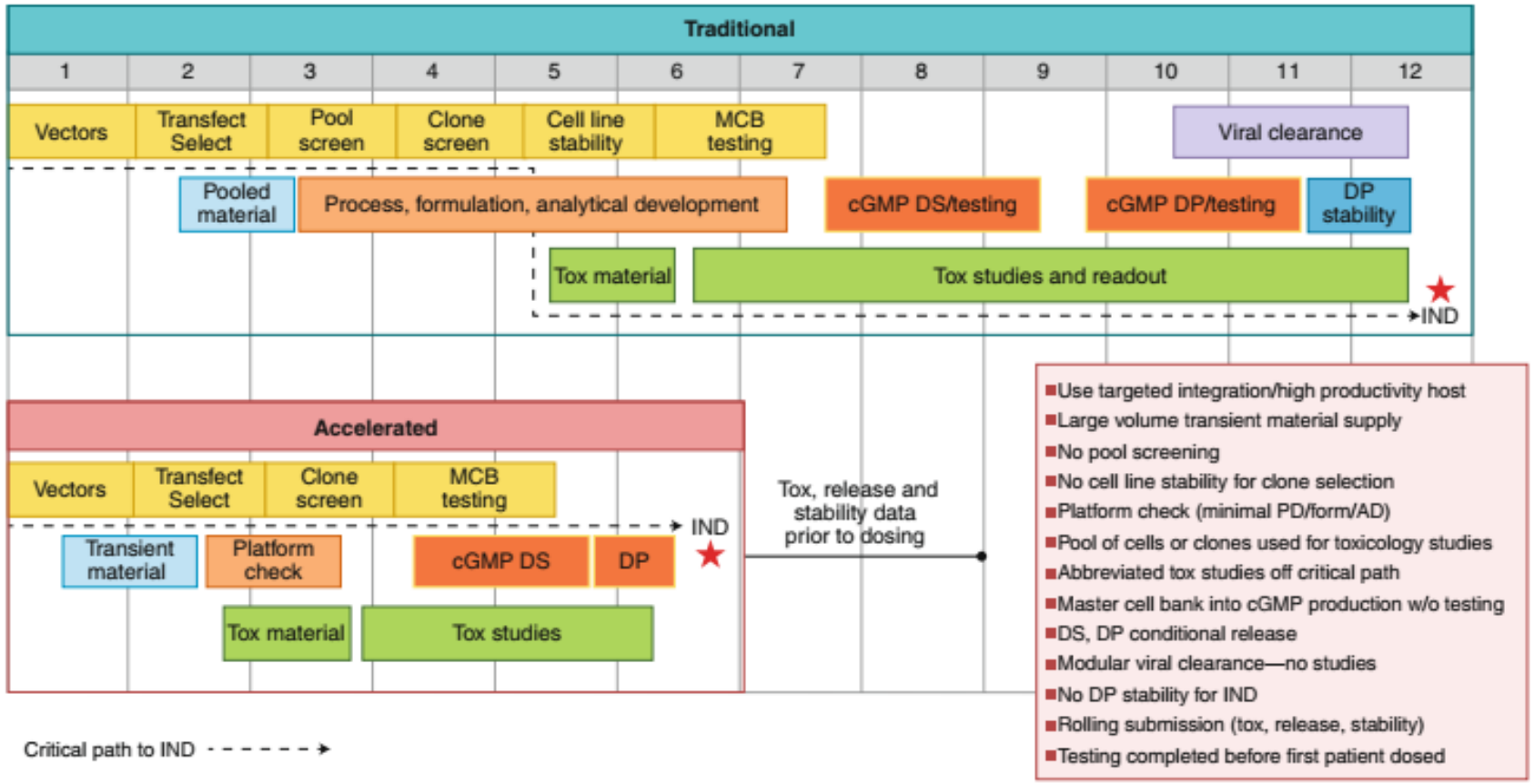
- The primary antigenic epitope is the S protein
- S- protein facilitates target cell binding and fusion after binding the angiotensin-converting enzyme 2 (ACE2) receptor on the cell surface
- ACE2 is found on cells in the respiratory system, gastrointestinal tract and endothelium

All antibodies designed to interact and engage S protein can neutralize the ability of the virus to bind and fuse with the target host cell



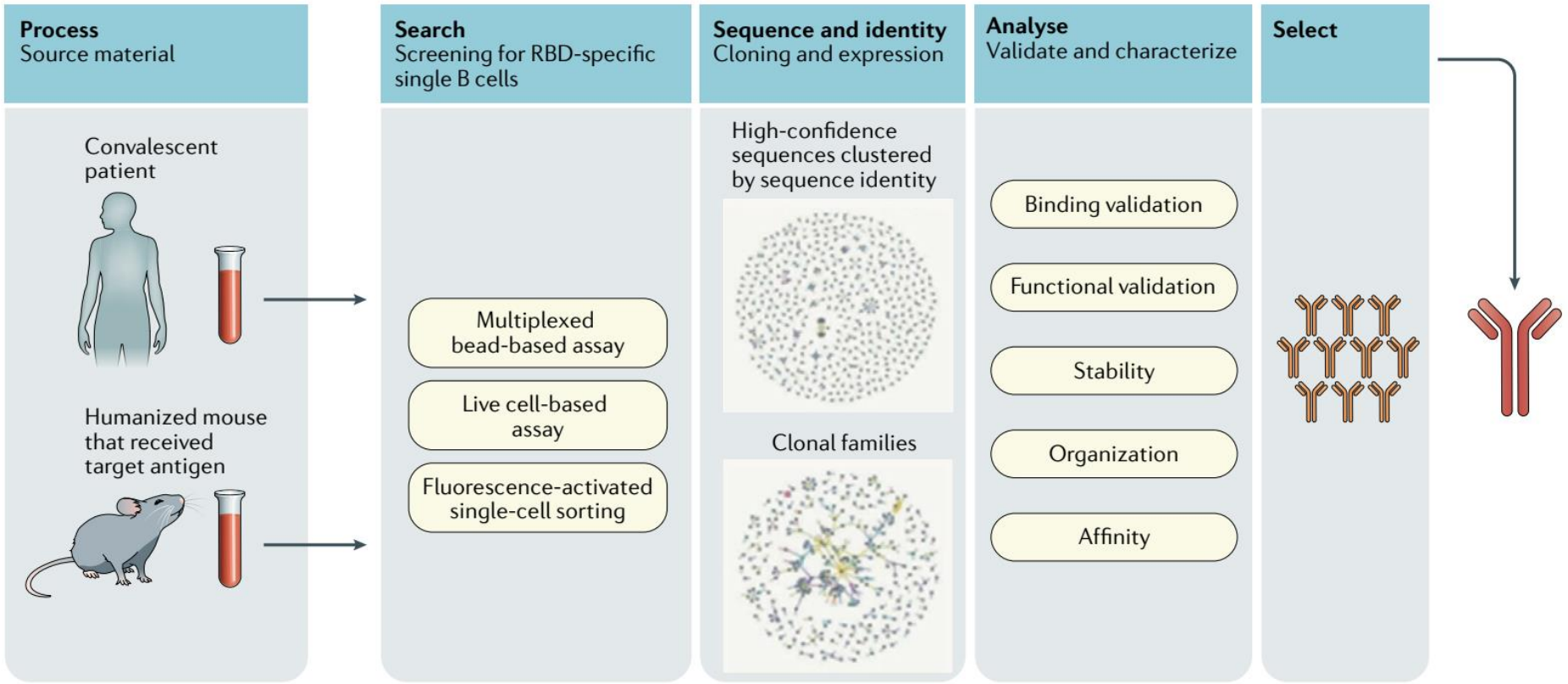
Adv Drug Deliv Rev **170** 113–141 (2021)

Monoclonal antibodies design “usual” vs. “COVID” timeline

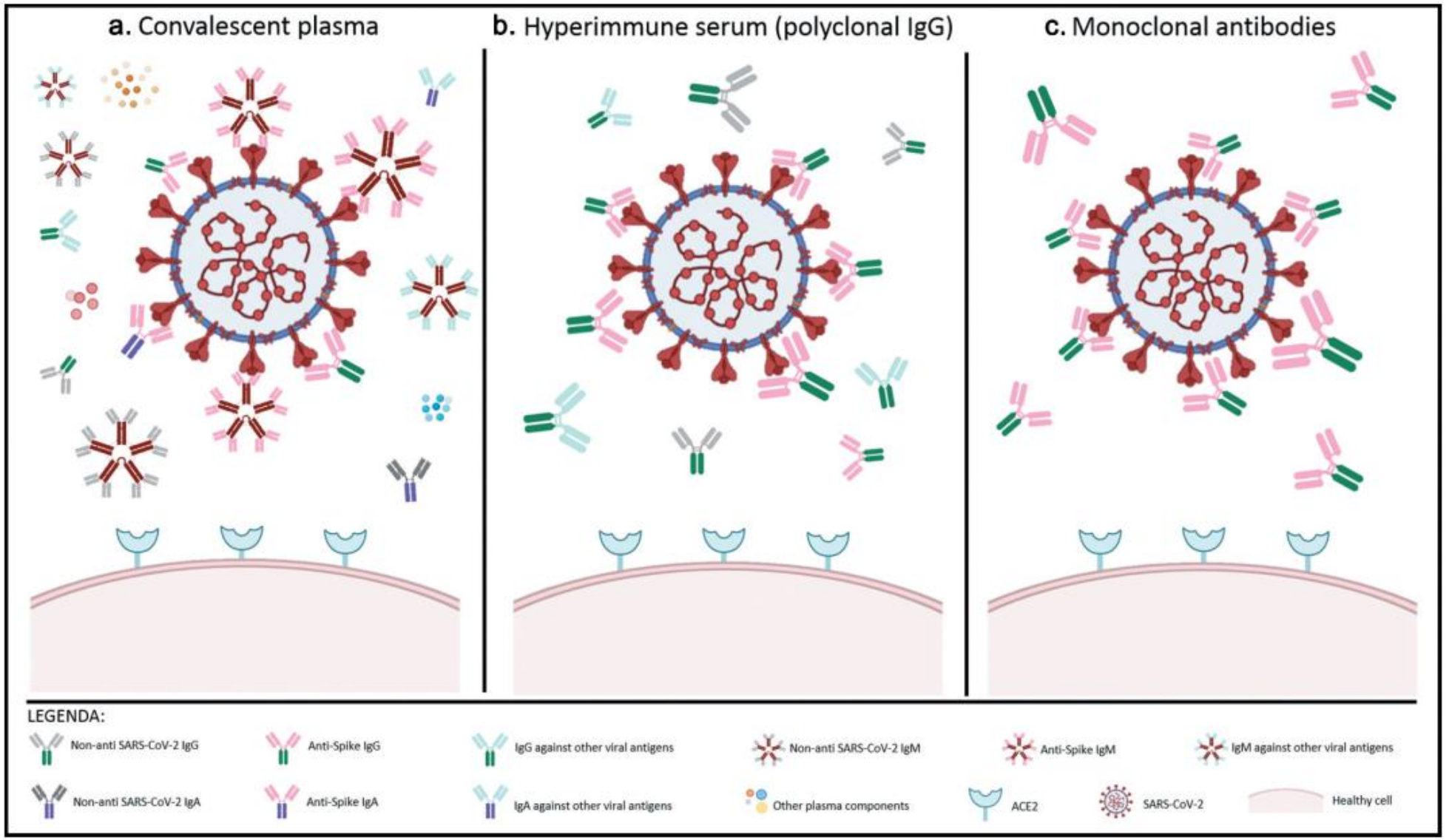


Nat Biotechnol **38,** 540–545 (2020)

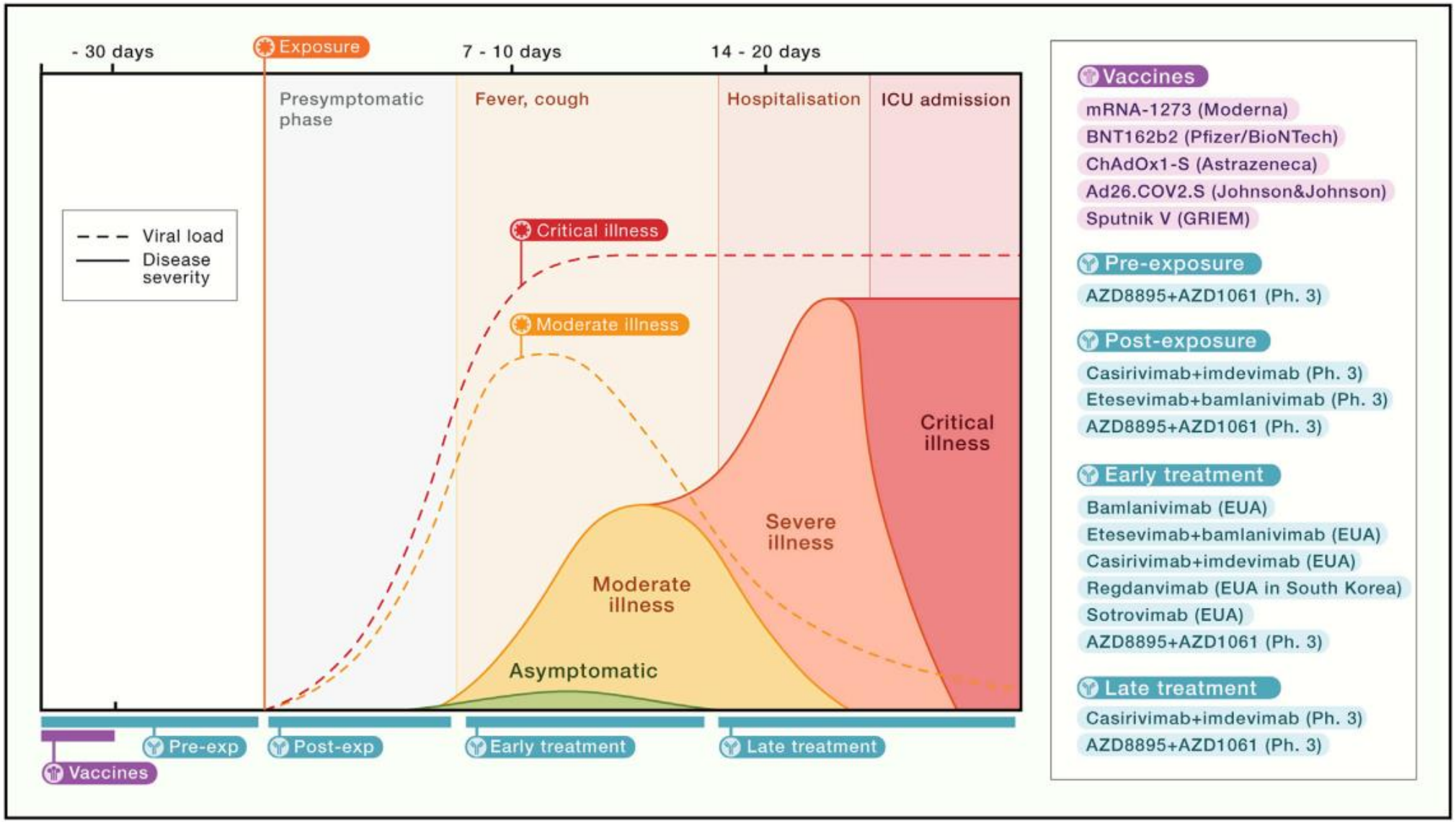
Easiest way to obtain anti-SARS-CoV-2 mAbs



Nat Rev Immunol **21,** 382–393 (2021)

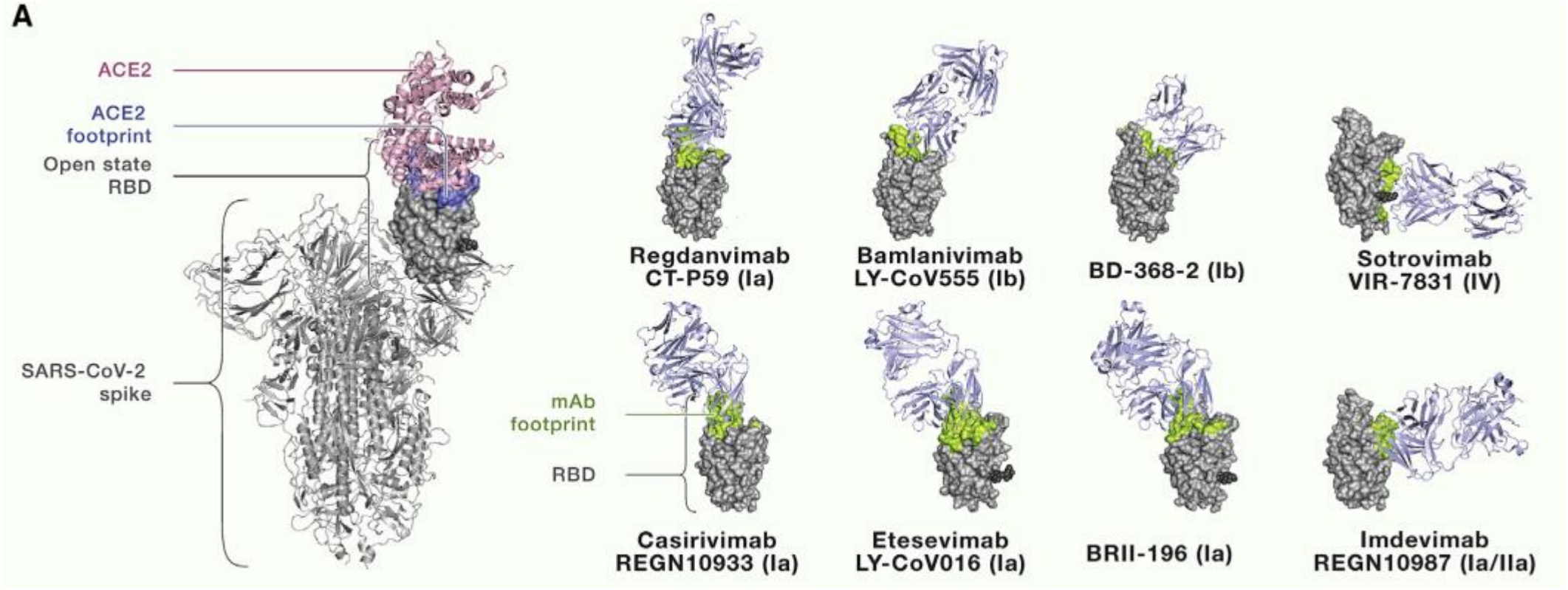


MABS **12** (1) e1854149 (2020)



Cell **184**, 10 June (2021)

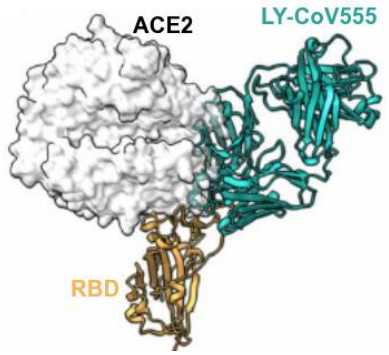
Where/how is mAb connected to SARS-CoV 2



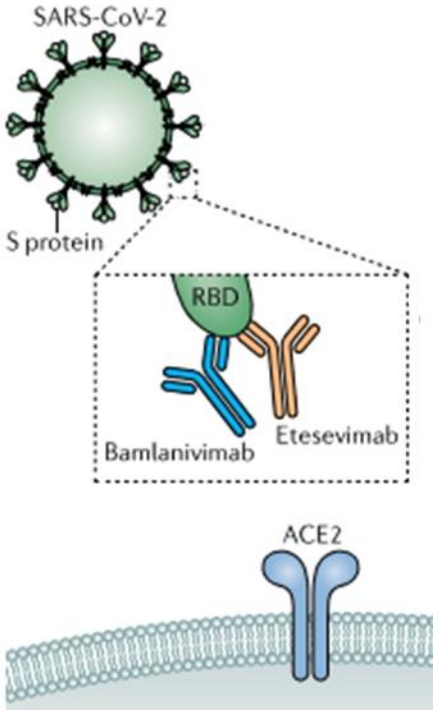
Cell **184**, 10 June (2021)

BAMLANIVIMAB & ETESEVIMAB

- Bamlanivimab (LY-CoV555) was derived from the **convalescent plasma** of a patient who had COVID-19
  - binds the S protein's RBD, engaging its cognate epitope in both up and down conformations, which makes this antibody potentially useful as a **monotherapy**
  - single N-linked glycosylation site on each heavy chain
  - unmodified in the Fc region
- Etesevimab (LY-CoV016) presents amino acid substitutions in the Fc region (L234A, L235A) to **reduce effector function**



LY-CoV016



16 March 2021
EMA/177113/2021
Committee for Medicinal Products for Human Use (CHMP)

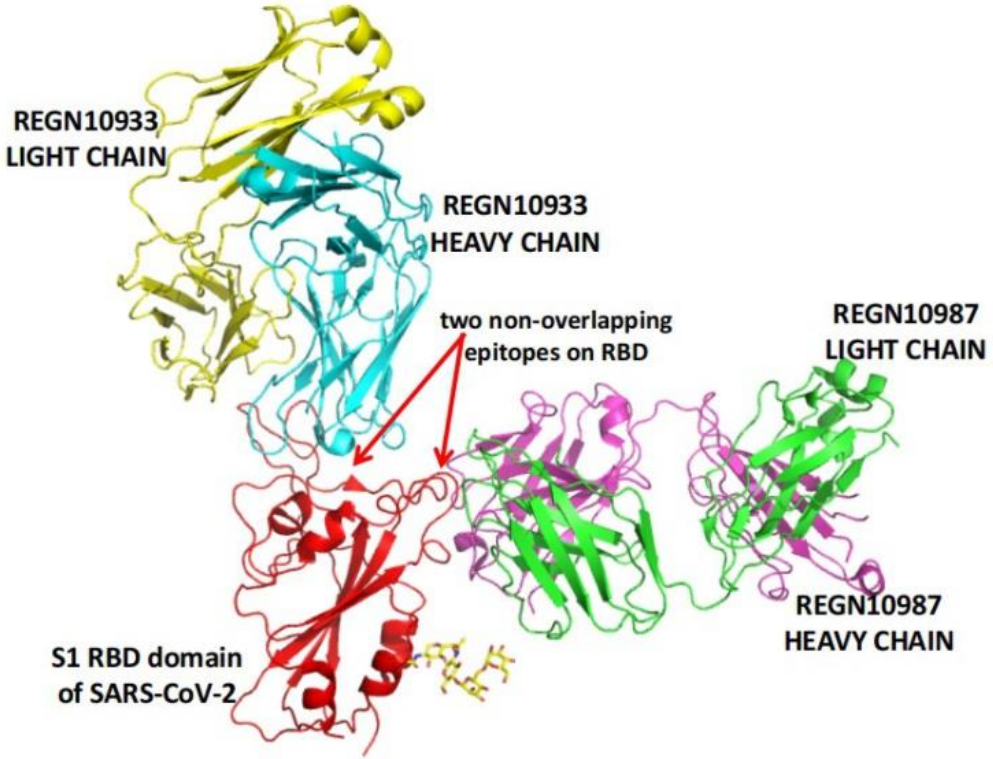
Pharmacol. Ther. **228** (107931) (2021)
http://opig.stats.ox.ac.uk/webapps/newsabdab/sabdab/

BAMLANIVIMAB & ETESEVIMAB

- Both exhibit neutralisation activity by blockage of the S protein interaction with angiotensin-converting enzyme 2 (ACE2) and prevent viral entry into human cells and viral replication targeting **overlapping**, **but different epitopes** of the RBD
- Produced in CHO cell lines
- Excipients/stabilizers include L-histidine buffer, sucrose, polysorbate 80, sodium chloride and WFI, and must be administered as a single intravenous infusion immediately after dilution with 0.9% sodium chloride

REGN-COV2 (Casirivimab & Imdevimab)

- **Pooled** from > 200 neutralizing mAbs derived from convalescent plasma and infected humanized mice
- Casirivimab (REGN10933) and Imdevimab (REGN10987) are **IgG1** recombinant monoclonal antibodies targeting non-overlapping epitopes of the RBD of the S protein and have **unmodified Fc regions** and both contain single N-linked glycosylation site on each heavy chain
- REGN-COV2 exhibits neutralisation activity by blockage of the S protein interaction with angiotensin-converting enzyme 2 (ACE2), antibody-mediated cytotoxicity and cellular phagocytosis in virally infected cells was shown *in vitro*



25 February 2021
EMA/142650/2021
Committee for Medicinal Products for Human Use (CHMP)

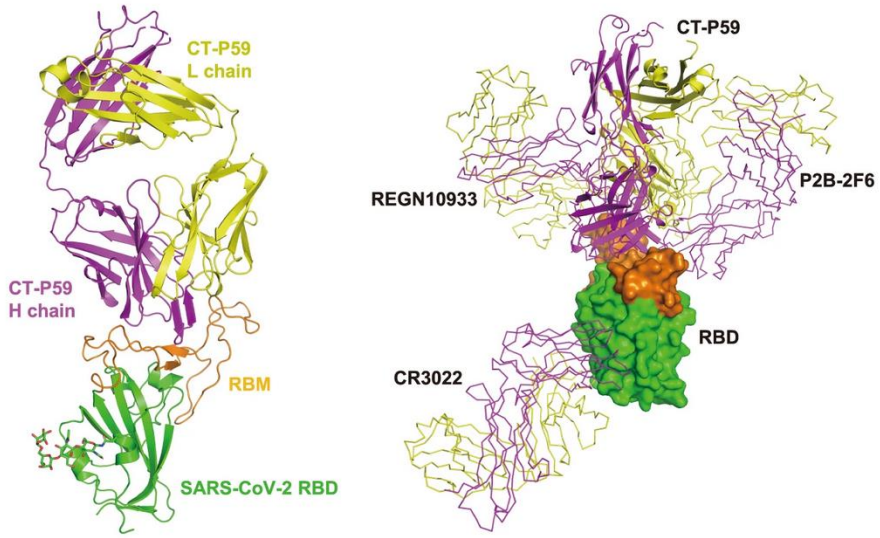
Int. J. Biol. Sci. **17** (2021)

REGN-COV2 (Casirivimab & Imdevimab)

- Produced in CHO cell line
- Excipients/stabilizers: L-histidine and L-histidine monohydrochloride monohydrate (target pH 6.0), sucrose, polysorbate 80 and WFI, and is administered together as a single intravenous infusion immediately after dilution with 0.9% sodium chloride

REGDANVIMAB

- Regdanvimab (CT-P59) recombinant human monoclonal IgG1 antibody targeting the RBD of the SARS-CoV-2 spike protein by blocking S protein interaction with ACE2
- contains a single N-linked glycosylation site on each heavy chain
- unable to mediate Fc-related activities



- binding orientation between CT-P59 and the RBD is different from other neutralizing mAbs
- CT-P59 can be a novel binder to the RBD
- CT-P59 has been proven to neutralize the D614G variant, one of the most infectious S protein's mutations

25 March 2021
EMA/192245/2021
Committee for Medicinal Products for Human Use (CHMP)

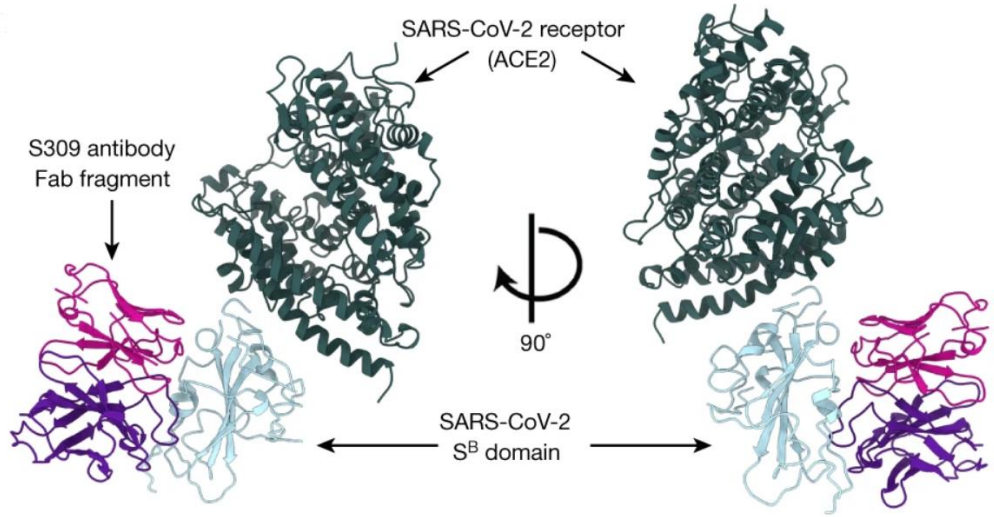
Nat Commun **12,** 288 (2021)

REGDANVIMAB

- Produced in a CHO cell line
- Excipients/stabilizers - L-histidine and L-histidine hydrochloride monohydrate, L-arginine, polysorbate 80 and WFI

SOTROVIMAB

- Sotrovimab (GSK4182136, VIR-7831) is an engineered human immunoglobulin monoclonal antibody of the IgG1κ subtype
- It is based on the identification and characterization of S309, an antibody identified from a **convalescent patient, who recovered from SARS-CoV in 2003**
- Sotrovimab binds to the SARS-CoV-2 RBD but can also engage in Fc-mediated receptor activities



20 May 2021
EMA/304600/2021
Committee for Medicinal Products for Human Use (CHMP)

Nature, **583**, 9 July (2020)

SOTROVIMAB

- Produced in CHO cell line
- Excipients/stabilizers include L-histidine and L-histidine monohydrochloride buffer, L-methionine buffer, sucrose, polysorbate 80 and WFI

Efficacy of neutralization vs. different strains of SARS-CoV-2

Cell **184,** June 10 (2021)

FORMULATION of mAbs

Antibody therapeutics are **large** (typically >150 kDa), **complex** in nature (most are glycosylated) and must be administered in **stoichiometric** rather than in catalytic quantities (nearly g/dose is required for many antibodies to be effective)

An initial formulation effort will likely employ an **isotonic**, **non- hemolytic**, **slightly acidic** solution that can be administered by ***intravenous*** infusion or ***subcutaneous*** injection in preclinical studies

Some stability information for the antibody drug can be obtained from initial *in silico* and *in vivo* testing during this preclinical phase that aids in the selection of a formulation that is used in clinical trials

FORMULATION of mAbs

Liquid sterile formulation

- Ready-to use
- Classical isotonic formulation with addition of stabilizers
- Less time to process

Lyophilized (freeze-dried) formulation

- Needs to be reconstituted
- Increased stability due to low water content
- Transport advantages

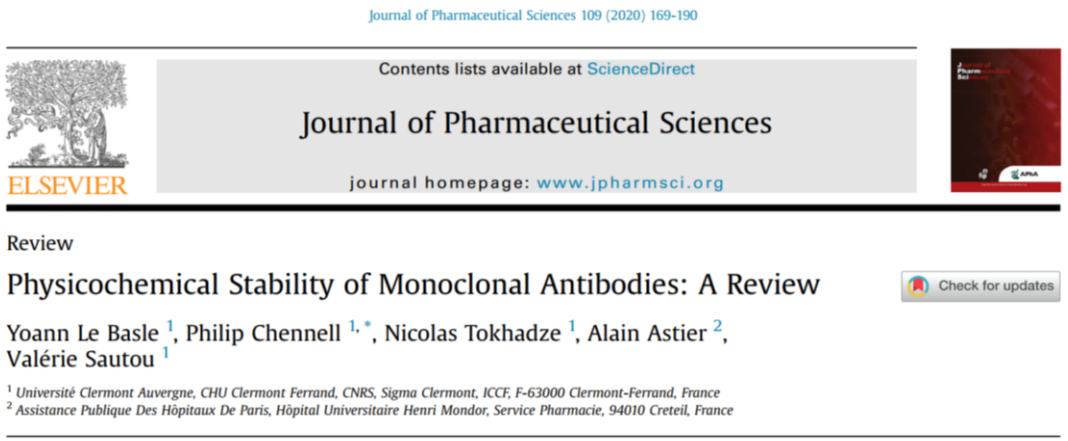
Stability of mAbs

Instability triggered by temperature change, pH change, water content, presence of salts and excipients, mechanical factors

mAbs instability may result in decrease of the biological activity and increase in immunogenicity

Physical instability
Aggregation
Surface adsorption
Denaturation

Chemical instability
Deamidation
Changes in disulphide bonds
Isomerisation
Oxydation
Hydrolisis
Fragmentation



Journal of Pharmaceutical Sciences 109 (2020) 169-190

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Review

Physicochemical Stability of Monoclonal Antibodies: A Review

Yoann Le Basle [1], Philip Chennell [1,*], Nicolas Tokhadze [1], Alain Astier [2], Valérie Sautou [1]

[1] Université Clermont Auvergne, CHU Clermont Ferrand, CNRS, Sigma Clermont, ICCF, F-63000 Clermont-Ferrand, France
[2] Assistance Publique Des Hôpitaux De Paris, Hôpital Universitaire Henri Mondor, Service Pharmacie, 94010 Creteil, France

Challenges in formulation of protein pharmaceuticals

- Production process design
- Selection of containers (glass and plastic surfaces adsorb proteins and peptides)
- Careful selection of excipients
  - High risk of aggregation
  - Potential loss of activity
- Need of sterility for parenterals
- Stability and shelf-life

What excipients are usually used?

- The basic amino acids histidine, lysine, and arginine stabilize all three domains of IgG
- Sugars and polyols are used as cryo-and lyoprotectants
- Surface active substances lower aggregation
- Different buffer mixtures are used in order to obtain desired pH/stability

	Frequency of Inclusion in MAb Formulations (%)		
Excipients	All	Lyophilized	Liquid
Polysorbate 80	57	45	62
Polysorbate 20	19	36	12
Poloxamer 188	3	0	4
Mannitol	8	9	8
Sorbitol	3	9	0
Sucrose	35	82	15
Trehalose	14	18	12
Dextrose	3	9	0
Dextran 40	3	9	0
NaCl	49	18	62
Arginine	8	0	12
Glycine	8	0	12
Methionine	3	0	4
Acorbic acid	3	0	4
NaOAc	3	9	0
Phosphate	32	27	35
Citrate	11	9	12
Acetate	16	0	23
Tris	3	0	4
Succinate	3	9	0
Histidine	35	27	38

https://bioprocessintl.com/manufacturing/formulation/rapid-formulation-development-for-monoclonal-antibodies/

Which excipients in anti- SARS-Cov-2 mAbs were used so far?

Anti-SARS-CoV2 mAbs	Excipients
Bamlanivimab and Etesevimab	L-histidine buffer, sucrose, polysorbate 80, sodium chloride and WFI
Casirivimab & Imdevimab	L-histidine and L-histidine monohydrochloride monohydrate (target pH 6.0), sucrose, polysorbate 80 and WFI
Regdanvimab	L-histidine and L-histidine hydrochloride monohydrate, L-arginine, polysorbate 80 and WFI
Sotrovimab	L-histidine and L-histidine monohydrochloride buffer, L-methionine buffer, sucrose, polysorbate 80 and WFI

What future holds?

- The approved agents are all conventional full-length IgG
- More than 50 mAbs are still in clinical trials
- Apart from the antibody format, engineering has been accomplished in the natural isolates in different ways, e.g., the LALA mutation was introduced into the Fc portion of CB6 to lower the risk of Fc-mediated acute lung injury
- It is possible for IgM/IgA, IgY based therapeutics, bi- or tri-specific antibodies, singledomain- derived from phage display libraries, fusion proteins, etc. to soon progress in clinical trials
- Potential combining neutralizing mAb with the catalytic activity of Cas13, a fusion-protein targeting, and cutting the viral RNA (AntiBody And CAS fusion (ABACAS)), so that agents would exert both a prophylactic and anti-viral effect

HVALA NA PAŽNJI